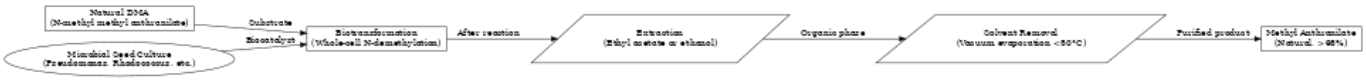
Production of Natural Methyl Anthranilate via Microbial N-Demethylation

Flow Diagram:



Lab-Scale Protocol (10 g Target Yield):

Materials Needed:

- N-Methyl methyl anthranilate (DMA): 12.5 g (0.083 mol)
- Microbial strain: Pseudomonas putida or Rhodococcus sp.
- Media: LB or minimal medium + glucose
- Phosphate buffer: pH 7.0
- Ethyl acetate (natural compliant)
- Na2SO4 (anhydrous)

Step-by-Step Instructions:

1. Seed Culture Preparation:
   - Inoculate microbe into 250 mL LB medium in a 1 L flask.
   - Incubate at 28-30 degreesC, 200 rpm for 16 hours (OD600 ~1.0).

2. Main Reaction Setup:
   - In 2 L flask: 1.5 L phosphate buffer (50 mM, pH 7.0)
   - Add 20 g glucose, 150 mL seed culture, and 12.5 g DMA.
   - Incubate at 30 degreesC, 200 rpm for 24-36 hours.

3. Monitoring:
   - Take samples every 6 hours. Extract with ethyl acetate.
   - Analyze via GC-MS or HPLC.

4. Extraction:
   - Add 500 mL ethyl acetate. Shake and separate layers.
   - Wash organic layer with brine, dry over Na2SO4, filter.

5. Concentration:

- Evaporate under vacuum at <50 degreesC. Expect ~10 g crude MA.

6. Purification (Optional):

- Distill under reduced pressure or purify with chromatography.

7. Quality Check:

- Confirm purity >98% by GC-MS.